

**IN THE SPECIFICATION:**

Please amend the specification as follows:

Please replace the following paragraphs:

Page 1, first paragraph after the heading, "CROSS-REFERENCE TO RELATED APPLICATION"

This application is a continuation-in-part of U.S. patent application Serial No. 08/320,982, filed October 11, 1994, **now U.S. Patent No. 5,801,026**, which itself is a continuation-in-part of U.S. patent application Serial No. 08/314,596, filed September 26, 1994, which is **U.S. Patent No. 5,668,292**, now abandoned. The entire contents of U.S. patent application Serial No. 08/320,982, **now U.S. Patent No. 5,801,026** and U.S. patent application Serial No. 08/314,596, **which is U.S. Patent No. 5,668,292** are hereby incorporated by reference and relied upon.

Page 2, second paragraph, after the heading, "BACKGROUND"

Extensive surveys of the fatty acid composition of seed oils from different species of higher plants have resulted in the identification of at least 33 structurally distinct monohydroxylated plant fatty acids, and 12 different polyhydroxylated fatty acids that are accumulated by one or more plants species (reviewed by van de Loo *et al.* 1993). Ricinoleic acid, the principal constituent of the seed oil from the castor plant *Ricinus communis* (L.), is of commercial importance. We have previously described the cloning of a gene from this species that encodes a fatty acid hydroxylase, and the use of this gene to produce ricinoleic acid in transgenic plants of other species (see U.S. patent application Serial No. 08/320,982, filed October 11, 1994, **now U.S. Patent No. 5,801,026**). The scientific evidence supporting the claims in that patent application were subsequently published (van de Loo *et al.*, 1995). The use of the castor hydroxylase gene to also produce other hydroxylated fatty acids such as lesquerolic acid, densipolic acid, hydroxypalmitoleic, hydroxyerucic and auricolic acid in transgenic plants is the subject of this invention. In addition, the identification of a gene encoding a homologous

hydroxylase from *Lesquerella fendleri*, and the use of this gene to produce these hydroxylated fatty acids in transgenic plants is the subject of this invention.

Pages 8-9, third paragraph, after the heading, "Conceptual basis of the invention"

In U.S. patent application No. 08/320,982, now U.S. Patent No. 5,801,026, we described the use of a cDNA clone from castor for the production of ricinoleic acid in transgenic plants. As noted above, biochemical studies by others had suggested that the castor hydroxylase may not have strict specificity for oleic acid but would also catalyze hydroxylation of other fatty acids such as icosenoic acid (20:1<sup>cis $\Delta$ 11</sup>) and erucic acid (13-docosenoic acid; 22:1<sup>cis $\Delta$ 13</sup>) would be expected to accumulate some of the hydroxylated derivatives of these fatty acids due to the activity of the hydroxylase on these fatty acids. We have now obtained additional direct evidence for such a claim based on the production of ricinoleic, lesquerolic, densipolic and auricolic fatty acids in transgenic Arabidopsis plants and have included such evidence herein as Example 1.

Pages 9-11, second paragraph,

In view of the high degree of sequence similarity between  $\Delta$ 12 fatty acid desaturases and the castor hydroxylase (van de Loo *et al.*, 1995), the validity of claims for the use of desaturase or hydroxylase genes or sequences derived therefrom for the identification of genes of identical function from other species must be viewed with skepticism. In this application, we teach a method by which hydroxylase genes can be distinguished from desaturases and describe methods by which  $\Delta$ 12 desaturases can be converted to hydroxylases by the modification of the gene encoding the desaturases. A mechanistic basis for the similar reaction mechanisms of desaturases and hydroxylases was presented in the earlier patent application (No. 08/320,982, now U.S. Patent No. 5,801,026). Briefly, the available evidence suggests that fatty acid desaturases have a similar reaction mechanism to the bacterial enzyme methane monooxygenase which catalyses a reaction involving oxygen-atom transfer ( $\text{CH}_4 \rightarrow \text{CH}_3\text{OH}$ ) (van de Loo *et al.*, 1993). The cofactor in the hydroxylase component of methane monooxygenase is termed a  $\mu$ -oxo bridged diiron cluster (FeOFe). The two iron atoms of the FeOFe cluster are liganded by protein-derived nitrogen or oxygen atoms, and are tightly redox-coupled by the covalently-

bridging oxygen atom. The FeOFe cluster accepts two electrons, reducing it to the diferrous state, before oxygen binding. Upon oxygen binding, it is likely that heterolytic cleavage also occurs, leading to a high valent oxoiron reactive species that is stabilized by resonance rearrangements possible within the tightly coupled FeOFe cluster. The stabilized high-valent oxoiron state of methane monooxygenase is capable of proton extraction from methane, followed by oxygen transfer, giving methanol. The FeOFe cofactor has been shown to be directly relevant to plant fatty acid modifications by the demonstration that castor stearyl-ACP desaturase contains this type of cofactor (Fox *et al.*, 1993).